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PHYTOCHEMICAL AND THIN LAYER CHROMATOGRAPHIC PROFILE OF DIFFERENT EXTRACTS FROM WHOLE AERIAL PART- *ARGYREIA NERVOSA*

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ABSTRACT

The present studies aimed at the phytochemical and thin layer chromatographic profile conducted on different extracts from whole aerial part of *Argyreia nervosa* (Burm.f.) Bojer**,** a woody climber, distributed throughout India. The crude powder drug, ethyl acetate and methanol extracts are investigated for the presence of different phytochemicals. Results show the presence of some major phytochemicals like alkaloids, glycosides, tannins and flavonoids. Thin layer chromatography provides the nature of phytochemicals present in the respective extracts. The results of this study will possibly prove useful for establishing scientific standards for the identification of nature of the phytochemicals present in it.

Key words: *Argyreia nervosa*, *Argyreia speciosa*, *Convolvulaceae*, Phytochemistry, Thin Layer Chromatography (TLC), Aerial Part.

INTRODUCTION

The use of plants as medicine is as old as human civilization. People of all ages in both developing and developed countries use plants in an attempt to cure various diseases and to get relief from physical sufferings. Natural products are a source for bioactive compounds and have potential for developing some novel therapeutic agents.

Argyreia nervosa (Burm.f.) Bojer synonym *Argyreia speciosa* (USDA, 2011) belongs to family *Convolvulaceae* (USDA, 2011) is a Vine Forb/herb (USDA, 2011). In hindi it is known as samundar-ka-pat (Anonymous, 1995). It is distributed throughout India, up to an altitude of 300 m, often cultivated native in India from Assam and Bengal to Karnataka (Anonymous, 1985; Guhabakshi *et al*., 1999*;* Nadkarni, 1976). Leaves are 7.5-3.0 by 6.3-2.5 cm. (sometimes even larger), ovate, acute glabrous above, persistently white-tomentose beneath, base cordate; petioles 5-15 cm. long, white-tomentose, characteristic odour and slightly bitter taste (Anonymous, 1985; Kirtikar and Basu, 1981). Stem stout, white tomentose, characteristic odour and slightly bitter taste (Kirtikar and Basu, 1981).

Reports on the benefits of *Argyreia nervosa* are rare compared to that of other species, probably due to the difficulty in identifying the material or due to lack of sufficient reported material. Hence, the objective of the present study is to collect data on the phytochemical and thin layer chromatographic profile.

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MATERIALS AND METHODS

Collection, Authentication and Preparation of Plant Material

The fresh Aerial part collected from local area of Barpali, (Dist-Bargarh, Orissa). The plant was

authenticated by Botanical Survey of India (BSI), Central National Herbarium Howrah, Kolkata, India. Ref. no. CNN/I-I/49/2010/Tech.II/285. The whole aerial part was dried under shade and powdered by the help of mechanical process. Powder of whole aerial part was stored in a suitable place.

Extraction

The dried powder plant material was extracted with ethyl acetate and methanol, by successive cold maceration method with increasing order of their polarity. The powdered drug was extracted for 7 days with each solvent. The extract was then filtered using filter paper and the filtrate so obtained was evaporated in a distillation unit (Harborne, 1998).

Phytochemical Profile

Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, proteins, saponins and glycosides were carried out on powder drug and extracts using standard procedure (Shah and Nayak, 2008; Kokate *et al*., 2002).

Thin Layer Chromatographic (TLC) Profile

The ethyl acetate and methanol extracts of the *Argyreia nervosa* were subjected to thin layer chromatographic analysis, to find the presence of number of chemical constituents to support the chemical test as well as chemical nature.

Analytical TLC plates were prepared by pouring the silica gel G slurry on the glass plates. Prepared plates then subjected to dryness for 30 minutes in air and then in an oven at 110^0C for another 30 minutes. The ethyl acetate and methanol extracts were applied as a single spot at 2 cm from the edge so that the solvent level will be at least 1cm below the center of the spot, keeping the spot small by using capillary tubes and allowed to dryness at room temperature. Solvent system was taken by considering the phytochemical study with reference to (Bobbitt JM, 1966). The TLC plate after spotting of the sample was placed vertically inside the development chamber according to ascending technique, previously being saturated with the solvent system. Then the lid was closed and the TLC plate was developed up to $3/4^{th}$ of its length. The plate was then dried at room temperature by keeping on a flat surface. The colored substances marked which were visual on the TLC. Colorless components were detected by using visualizing agent, iodine vapours and under UV light (Bobbitt JM, 1966; Stahl E, 2005; Wagner H and Bladt S 2002).

RESULTS

Extraction

The dried powder of whole aerial part of *Argyreia nervosa* was extracted with ethyl acetate, methanol by successive cold maceration method. The ethyl acetate and methanol extracts so obtained having yield 3.57% w/w and 4.93% w/w respectively and a general study reveal yield, consistency and color of extracts given in Table 1.

Phytochemical Profile

Preliminary phytochemical study reveals that the powder drug shows presence of almost all secondary metabolite, Ethyl acetate extract of whole aerial part from *Argyreia nervosa* shows the presence of fixed oil, fats, phytosterols, glycosides, flavonoids, alkaloids, tannins and phenolic compounds while methanol extract shows the presence of carbohydrates, protein, amino acids, fixed oil, fats, phytosterols, glycosides, flavonoids, alkaloids, tannins and phenolic compounds a brief study is given in Table 2.

Thin Layer Chromatographic (TLC) Profile

The ethyl acetate and methanol extracts of the *Argyreia nervosa* were subjected to thin layer chromatographic analysis. The qualitative evaluation of the plate was done by determining the migrating behavior of the separated substances given in the form of R*f* values (Stahl E, 2005; Wagner H and Bladt S 2002). Resulting R*f* are given in Table 3 & Table 4. The R*f* value is the "retention factor" or the "ratio-to-front" value expressed as a decimal fraction.

The R*f* value can be calculated as:

$$Rf = \frac{\text{Distance travelled by solute front}}{\text{Distance travelled by solvent front}}$$

Table 1. Yield, color and consistency of Extracts

Extracts	%age Yield (w/w)	Consistency	Color	Color under UV
Ethyl acetate	3.57%	Sticky	Greenish black	Brown
Methanol	4.93%	Greasy	Dark black	Dark brown

Table 2. Phytochemical Profile of *Argyreia nervosa.*

TEST/REAGENT USED	POWDERED DRUG	ETHYL ACETATE EXTRACT	METHANOL EXTRACT
1.TEST FOR CARBOHYDRATES			
Molisch's test	+ve	+ve	+ve
Fehling's Test	+ve	-ve	+ve
Benedict's Test	+ve	-ve	+ve
Barfoed's Test	+ve	-ve	+ve
Tollen's phluroglucinol test	+ve	-ve	+ve
Seliwanoff's test	-ve	-ve	-ve
Test for Starch	-ve	-ve	-ve
2.TEST FOR GUMS & MUCILAGE			
Swelling index	-ve	-ve	-ve
3.TEST FOR PROTEINS & AMINO ACIDS			
Ninhydrin test	-ve	-ve	-ve
Biuret Test	+ve	+ve	+ve
Tannic acid test	-ve	-ve	-ve
Millon's Test	-ve	-ve	+ve
Xanthoprotein Test	+ve	+ve	+ve
4.TEST FOR FIXED OILS & FATS			
Spot Test	+ve	+ve	+ve
5.TEST FOR PHYTOSTEROLS			
Liebermann-Burchard	+ve	+ve	+ve
Salkowski's test	+ve	+ve	+ve
6.TEST FOR GLYCOSIDES			
Baljet's test	+ve	+ve	+ve
Legal's Test	+ve	+ve	+ve
Borntrager's test	-ve	-ve	-ve
Modified Borntrager's	-ve	-ve	-ve
7.TEST FOR SAPONIN			
Foam test	+ve	-ve	-ve
8.TEST FOR FLAVONOIDS			
Shinoda test	+ve	+ve	+ve
Lead acetate test	+ve	+ve	+ve
Fluorescence test	+ve	+ve	+ve
Action of alkali and acid	+ve	+ve	+ve
9.TEST FOR TANNINS AND PHENOLIC COMPOUNDS			
Ferric chloride test	+ve	+ve	+ve
10.TEST FOR ALKALOIDS			
Mayer's test	+ve	+ve	+ve
Dragendorff's test	+ve	+ve	+ve
Wagner's test	+ve	+ve	+ve
Hager's test	+ve	+ve	+ve

+ ve Present, -ve Absent

Table 3. TLC Profile of Ethyl Acetate Extract

S.No.	Coating Material	Solvent System	Detecting Reagents	Spots	R*f* Values	Inference
1.	Silica Gel G	Benzene:Chloroform:Ethanol 2.85:5.7:1.45	U.V Long wavelength	1	0.80	May be ergometrine
2.	Silica Gel G	Benzene:Chloroform:Ethanol 2.85:5.7:1.45	U.V Short Wavelength	3	0.96,0.91 0.80	May be ergometrine
3.	Silica Gel G	Choloroform:Glacial Acetic Acid 9.5:0.5	U.V	1	0.68	May be indole derivatives
4.	Silica Gel G	Ethyl Acetate:Formicacid:Glacial Acetic Acid:Water 10:1.1:1.1:2.6	U.V	1	0.89	May be Quercetin and kaempferol
5.	Silica Gel G	Chloroform:Methanol 9.5:0.5	Dragendorff's reagent	2	0.92,0.54	May be alkaloid
6.	Silica Gel G	Methanol	U.V	1	0.77	May be flavanoids
7.	Silica Gel G	Chloroform:Ethanol 1:1	U.V	1	0.74	May be isoflavones
8.	Silica Gel G	Benzene:Methanol 8:2	Dragendorff's reagent	3	0.96,0.470.27	May be opium alkaloid
9.	Silica Gel G	Hexane:Ethyl Acetate 5:2	U.V	2	0.88,0.76	May be cumarin
10.	Silica Gel G	Chloroform:Ethanol 9:1	Dragendorff's reagent	2	0.89,0.56	May be morphine alkalods
11.	Silica Gel G	Acetic Acid:Water 9:1	Iodine chamber	1	0.90	May be sterols and sterol acetate
12.	Silica Gel G	Cyclohexane:EthylAcetate:Water 6:4:0.01	50% Sulphuric Acid	2	0.89,0.81	May be Sterol and sterol dervatives
13.	Silica Gel G	Pet. Ether:EthylAcetate:Benze 8.5:1.0:0.5	U.V	2	0.24,0.14	May be β-sitosterol
14.	Silica Gel G	Pet. Ether:EthylAcetate:Benze 8.5:1.0:0.5	Iodine chamber	8	0.85,0.77 0.70,0.53 0.44,0.33 0.25,0.16	May be β-sitosterol
15.	Silica Gel G	Benze:Ethyl Acetate 9:1	U.V	2	0.81,0.70	May be steroids
16.	Silica Gel G	Benze:Ethyl Acetate 9:1	Iodine Chamber	3	0.91,081 0.70	May be steroids
17	Silica Gel G	Cyclohexane:Ethyl Acetate 9:1	U.V	2	0.23,0.14	May be steroids
18.	Silica Gel G	Cyclohexane:Ethyl Acetate 9:1	Iodine chamber	8	0.92,0.69 0.63,0.49 0.41,0.29 0.23,0.14	May be steroids

Table 4. TLC Profile of Methanol Extract

S.No.	Coating Material	Solvent System	Detecting Reagents	Spots	R*f* Values	Inference
1.	Silica Gel G	Benzene:Chloroform:Ethanol 2.85:5.7:1.45	U.V Long wavelength	1	0.77	May be ergometrine
2.	Silica Gel G	Benzene:Chloroform:Ethanol 2.85:5.7:1.45	U.V Short Wavelength	2	0.22 0.77	May be ergometrine
3.	Silica Gel G	Choloroform:Glacial Acetic Acid 9.5:0.5	U.V	1	0.89	May be indole derivatives
4.	Silica Gel G	Ethyl Acetate:Formicacid:Glacial Acetic Acid:Water 10:1.1:1.1:2.6	U.V	1	0.92	May be Quercetin and kaempferol
5.	Silica Gel G	Chloroform:Methanol 9.5:0.5	Dragendorff's reagent	6	0.94,0.86 0.70,0.65 0.63,0.60	May be alkaloid
6.	Silica Gel G	Methanol	U.V	1	0.79	May be flavanoids
7.	Silica Gel G	Chloroform:Ethanol 1:1	U.V	1	0.71	May be isoflavones
8.	Silica Gel G	Benzene:Methanol 8:2	Dragendorff's reagent	1	0.53	May be opium alkaloid
9.	Silica Gel G	Hexane:Ethyl Acetate 5:2	U.V	3	0.90,0.85 0.40	May be cumarin
10.	Silica Gel G	Chloroform:Ethanol 9:1	Dragendorff's reagent	2	0.79,0.74	May be morphine alkalods
11.	Silica Gel G	Acetic Acid:Water 9:1	Iodine chamber	1	0.90	May be sterols and sterol acetate
12.	Silica Gel G	Cyclohexane:EthylAcetate:Water 6:4:0.01	50% Sulphuric Acid	2	0.89,0.83	May be Sterol and sterol dervatives
13.	Silica Gel G	Pet. Ether:EthylAcetate:Benze 8.5:1.0:0.5	U.V	2	0.25,0.20	May be β-sitosterol
14.	Silica Gel G	Pet. Ether:EthylAcetate:Benze 8.5:1.0:0.5	Iodine chamber	4	0.68,0.25 0.20,0.14	May be β-sitosterol
15.	Silica Gel G	Benze:Ethyl Acetate 9:1	U.V	4	0.81,0.72 0.67,0.44	May be steroids
16.	Silica Gel G	Benze:Ethyl Acetate 9:1	Iodine Chamber	5	0.86,0.81 0.72,0.67 0.44	May be steroids
17	Silica Gel G	Cyclohexane:Ethyl Acetate 9:1	U.V	2	0.30,0.23	May be steroids
18.	Silica Gel G	Cyclohexane:Ethyl Acetate 9:1	Iodine chamber	6	0.73,0.66 0.53,0.48 0.23,0.14	May be steroids

DISCUSSION

For the pharmacological as well as pathological discovery of novel drugs, the essential information's regarding the chemical constituents are generally provided by the qualitative phytochemical profile of plant extracts. Phytochemical studies play an important role in detecting the chemical compounds and biosynthetic origin (Harborne JB, 1998). The current trend of phytodrug based industry is to procure standardized extracts and related products of plants as raw materials. Therefore, phytochemical profile for bioactive extracts would be beneficial towards this end.

TLC profiling both extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals gives different R*f* values in different solvent system. This variation in R*f* values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in

selection of appropriate solvent system for separation of pure compounds by Column Chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the R*f* values of compounds in different solvent system. In the present state of affairs, TLC profiling of different extracts of whole aerial part in different solvent system indicated the presence of diverse type of phytochemicals in these plant. Different R*f* values of the compound also reflects an idea about their polariy. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

Present study after phytochemical studies concludes that there are various phytochemicals present in whole aerial part and different extracts from it. And TLC profiling of extracts in different solvent system confirms the presence of diverse group of phytochemicals and predict the nature of phytochemicals.

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